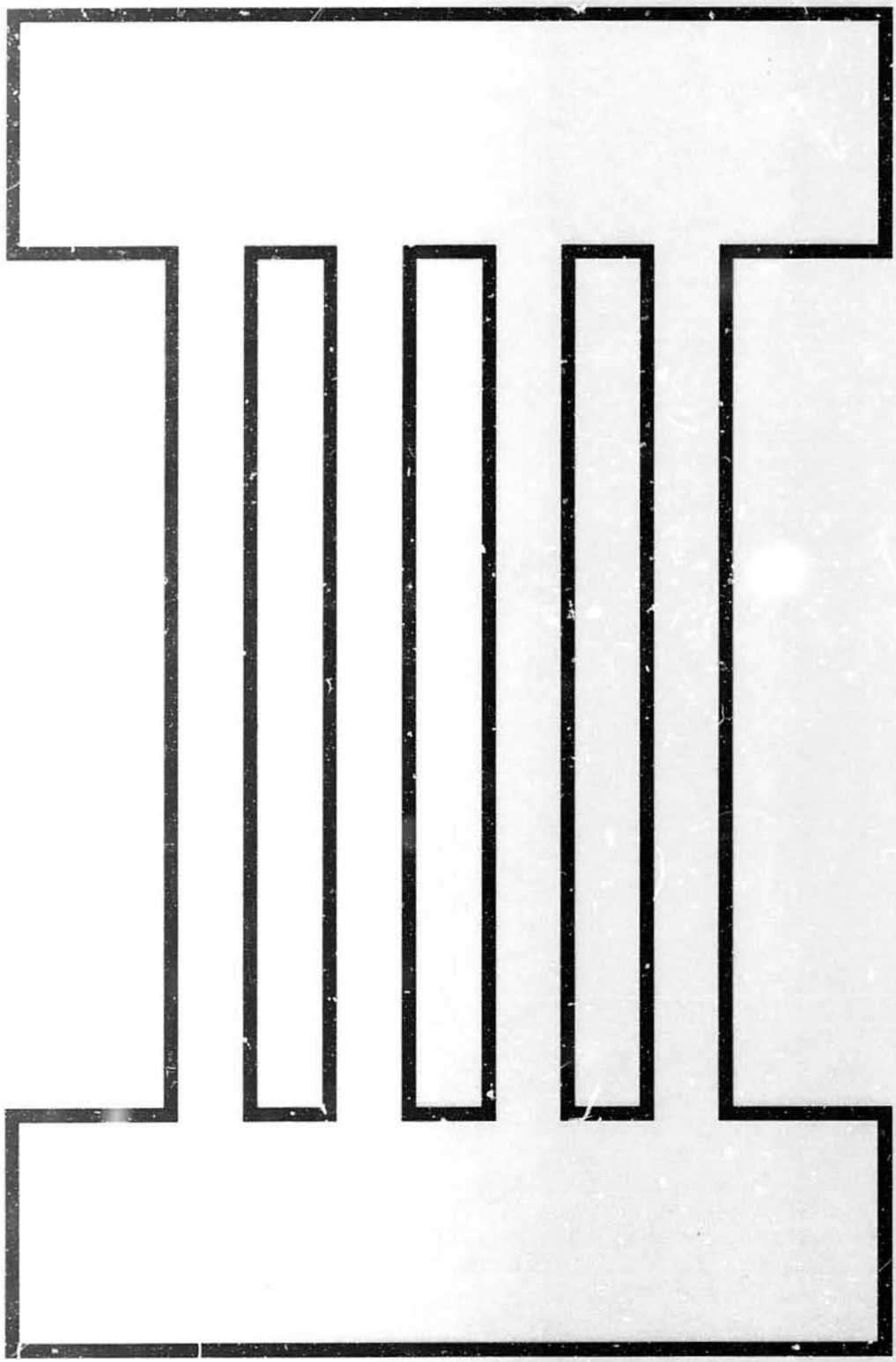
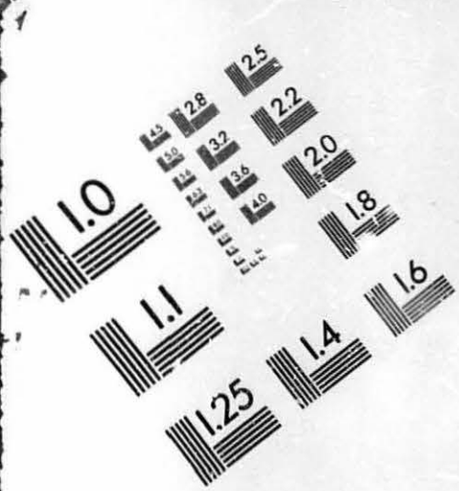
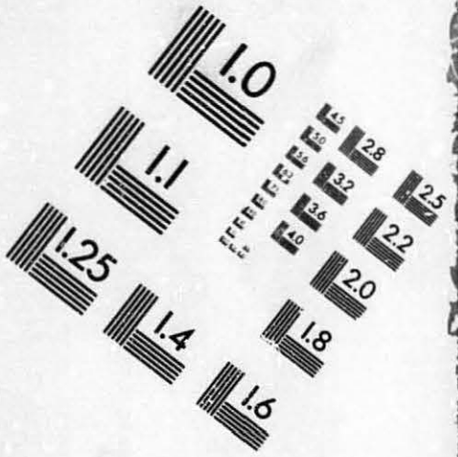
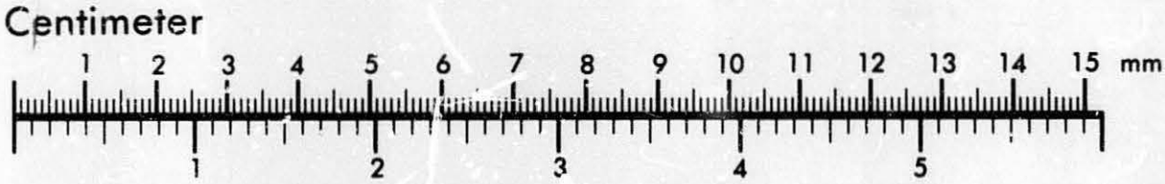
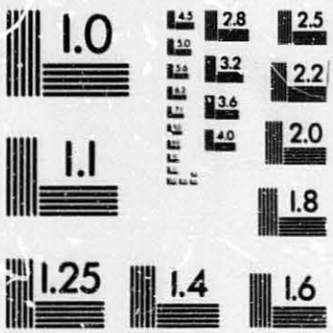


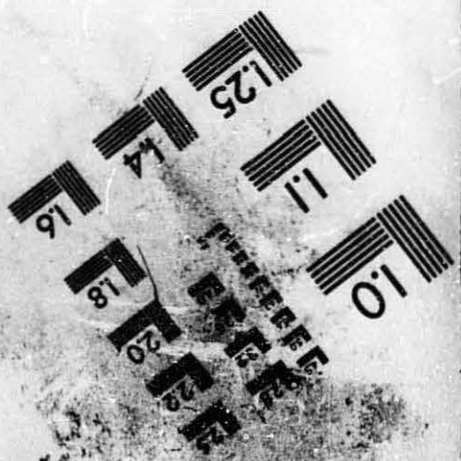




Association for Information and Image Management
1100 Wayne Avenue, Suite 1100
Silver Spring, Maryland 20910
301/587-8202







This file contains 619 pages

ADDENDUM TITLE PAGE

PMN FILE NUMBER 89-632

ADDENDUM LETTER C

DATE 8-23-91

THE BEST COPIES AVAILABLE WERE USED FOR THIS FILE

The documents included on this addendum fiche were received after the original PMN file was filmed.

Addenda are indexed and filmed several times a year. They are filed behind the original file fiche.

Please check with the [redacted] staff if the document you are looking for is not included in the PMN file or any of its addenda.

Addendum prepared by: CJG

Document Processing Center (DPC)
[redacted] Center (CBIC)
Information Control Section (ICS)
Chemical Information Branch (CIB)
Information Management Division (IMD)
Office of Toxic Subtances (OTS)

United States Environmental Protection Agency
Washington, DC 20460

EPA Memorandum of [redacted] Telephone Conversation

I. EPA Employee Identification

Name of Employee	Date
RONA BIRNBAUM	6/11/91
Organization	**Time**
NCB/CCD/OTS/OPTS	3:20

II. Second Party Identification

Call Is	Name	
☐ To ☑ From	[redacted]	EPA-OTS
[redacted]	Organization [redacted]	0007981897

50911001786

III. Concerning what TSCA CBI?

P89-632

IV. Content

[redacted] [redacted] [redacted] called for clarification of the protocal review letter.

I clarified that with EPA's comments, the protocal for the 90-day subchronic study are indeed acceptable and approved by EPA.

[redacted]

EPA Form 7740-17 (10-85)

000001

United States Environmental Protection Agency
Washington, DC 20460

EPA Memorandum of [redacted] Telephone Conversation

I. EPA Employee Identification

Name of Employee	Date
RONA BIRNBAUM	4/24/91
Organization	**Time**
NCB/CCD/OTS/OPTS	2:15

II. Second Party Identification

Call Is	Name	
☑ To ☐ From	[redacted]	
[redacted]	Organization	

III. Concerning what TSCA CBI?

P89-632

IV. Content

HERD has completed their review of the 28-day range finding study and concluded that the suggested dose levels of 25, 75, 250 mg/kg/day are acceptable for the 90-day study.

Although the submitted data reports the low-dose to be a NOEL, the serum enzyme changes + reduced body weight suggest some mild toxicity at this level.

EPA Form 7740-12 (10-85)

000002

United States Environmental Protection Agency
Washington, DC 20460

EPA Memorandum of TSCA CBI Telephone Conversation

I. EPA Employee Identification

Name of Employee	Date
RONA BIRNBAUM	5/29/90
Organization	**Time**
NCB/CCD/OTS/CPTS	11:45

II. Second Party Identification

Call Is	Name
☒ To ☐ From	[redacted]
Number	**Organization**
[redacted]	

III. Concerning what TSCA CBI?

P89-632 90-day protocol review issues

IV. Content

Per our conference call on 5/23/90, and his suggestion regarding sperm staging:

EPA will accept their suggested method for the high dose. All [redacted] animals must be tested. However, there is a caveat that if anything looks suspicious, unclear or if any deviations are seen in any sperm staging, then the middle doses must also be done.

Bonnie Stern & Jennifer Seed reviewed the method which you faxed to me.

EPA Form 7740-12 (10-85)

000003

United States Environmental Protection Agency
Washington, DC 20460

EPA Memorandum of TSCA CBI Telephone Conversation

I. EPA Employee Identification

Name of Employee	Date
Rona Birnbaum	5/7/90
Organization	**Time**
NCB/CED	4:50

II. Second Party Identification

Call Is:	Name
☒ To ☐ From	[redacted]
[redacted]	Organization [redacted]

III. Concerning what TSCA CBI?

P89-632

IV. Content

Regarding his question concerning a change in lab and the necessity for another protocal review, I explained that the protocal review is a qualitative one. Changing labs does not matter as long as the approved protocal with revisions is followed.

You should state that the protocal was followed in a cover letter when submitting the test data.

EPA Form 7740-12 (10-85)

000004

United States Environmental Protection Agency
Washington, DC 20460

EPA Memorandum of TSCA CBI Telephone Conversation

I. EPA Employee Identification

Name of Employee: Rona Birnbaum

Date: 3/9/90

Organization: EPA/OPTS/OTS/CCD/PNMB

Time: 10:35

II. Second Party Identification

Call is: ☒ To ☐ From

Name: [redacted]

Number: [redacted]

Organization: [redacted]

III. Concerning what TSCA CBI?

P89-632

IV. Content

I reviewed the MSDS with [redacted] from ETD and we offer the following comments:

1. Goggles requirement → use stronger language such as "must wear ...". This is based on the corrosivity of the substance.

2. "Washing thoroughly ..." should not be an afterthought. Bring this out a little more by moving the statement from "Other" to the first thing listed under workplace considerations.

The MSDS does cover all the requirements specified in the Order.

EPA Form 7740-12 (10-85)

000005

[redacted]

[redacted]

TD

[redacted]

April 16, 1991

R. B. 4/16/91

50-918000699

Ms. Rona Birnbaum
Office of Toxic Substances
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460

RE: PMN SUBSTANCE P-89-632

Dear Ms. Birnbaum:

On behalf of the [redacted], I am pleased to submit the final report of the two-week range finding study for PMN substance P-89-632. As we discussed, [redacted] would appreciate receiving EPA concurrence on the dose levels selected for the 90-day study which is being conducted pursuant to TSCA Section 5(e) Consent Order [redacted]

In the range-finding study three groups of animals were exposed for 14 days at dose levels of 1,500, 750 and 375 mg/kg/day. Deaths were reported at the dose levels of 1,500 and 750 mg/kg/day as well as other treatment related effects in surviving animals at these levels. The study report classifies the low dose of 375 mg/kg/day as the No Effect Level (NOEL) for this study.

In selecting dose levels for the 90-day study, we initially considered 375 as the upper dose. However, a careful analysis of the 14-day study suggests that although there were no deaths at this dose level early suggestions of toxicity were observed which cause us to believe that the 375 mg/kg/day level may be excessive as the high dose for the longer 90-day study. Specifically, there appears to be a decrease in body weight of more than 10% for two males at the 375 mg/kg/day level; thymic congestion was also reported for one of these males.

The TSCA guidelines for the 90-day oral toxicity study (40 CFR § 798.2650) states that the "highest dose level in rodents should result in toxic effects but not produce an incidence of fatalities which would prevent meaningful evaluation." Based on the reported findings in the 14-day study, we are concerned that dosing the animals at 375 mg/kg/day for 90 days may produce mortality and/or other toxic manifestations in excess of what is

---

[redacted]

generally considered acceptable for 90-day studies. It is therefore our intention to select 250 mg/kg/day as the upper dose for the study. We anticipate that this level will induce some adverse effects following 90 days of exposure and avoid loss of animals. For the low dose we are selecting 25 mg/kg/day to span one log unit and for the mid dose, 75 mg/kg/day, which is roughly halfway between the high and the low doses on a log scale.

Given that the 90-day study is being conducted pursuant to a TSCA Section 5(e) Consent Order we would like to receive the Agency's concurrence on these dose selections. Since [redacted] desires to start the 90-day study as soon as possible we would appreciate receiving a response from EPA within two weeks. Please let me know if you will be unable to respond within this time frame.

Sincerely,

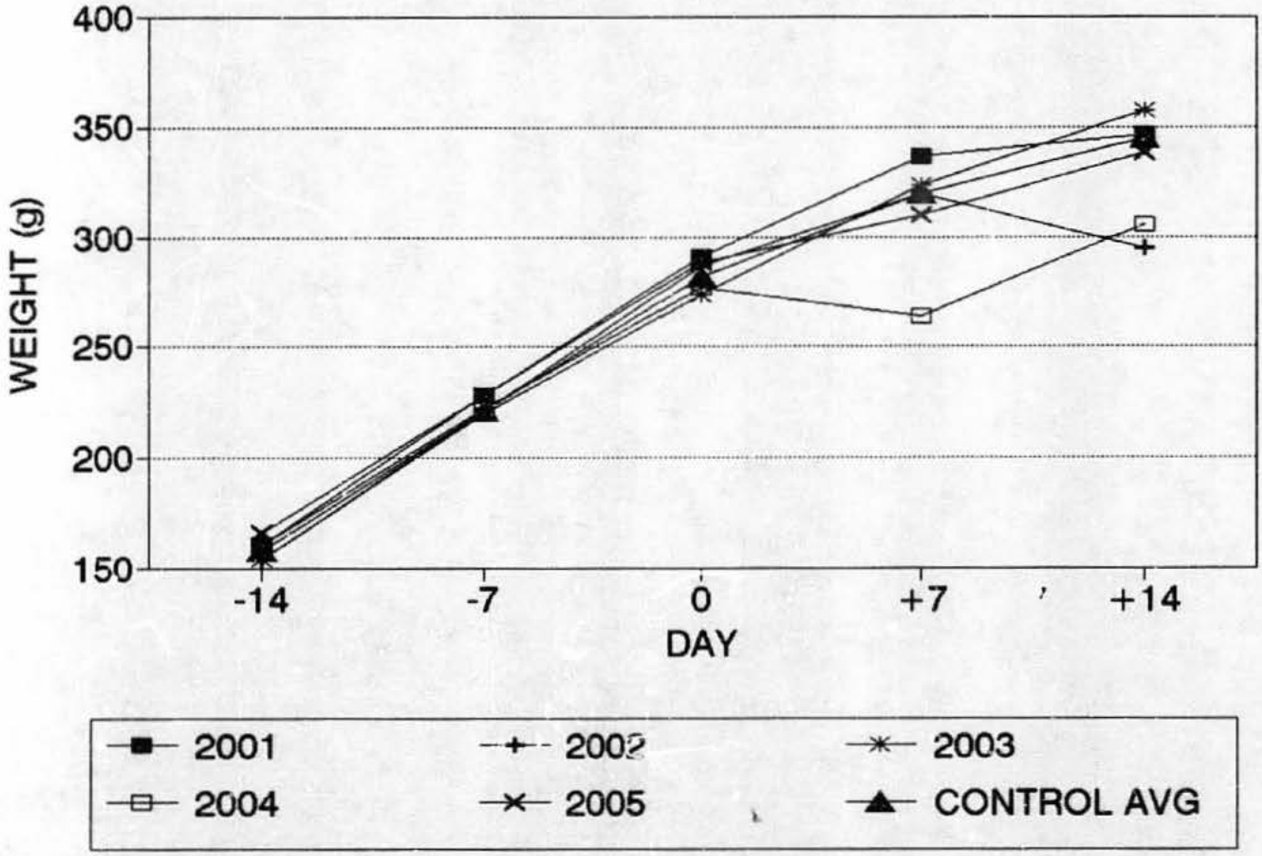
[redacted]

cc: [redacted] [redacted]

600007

MALE RAT BODY WEIGHT

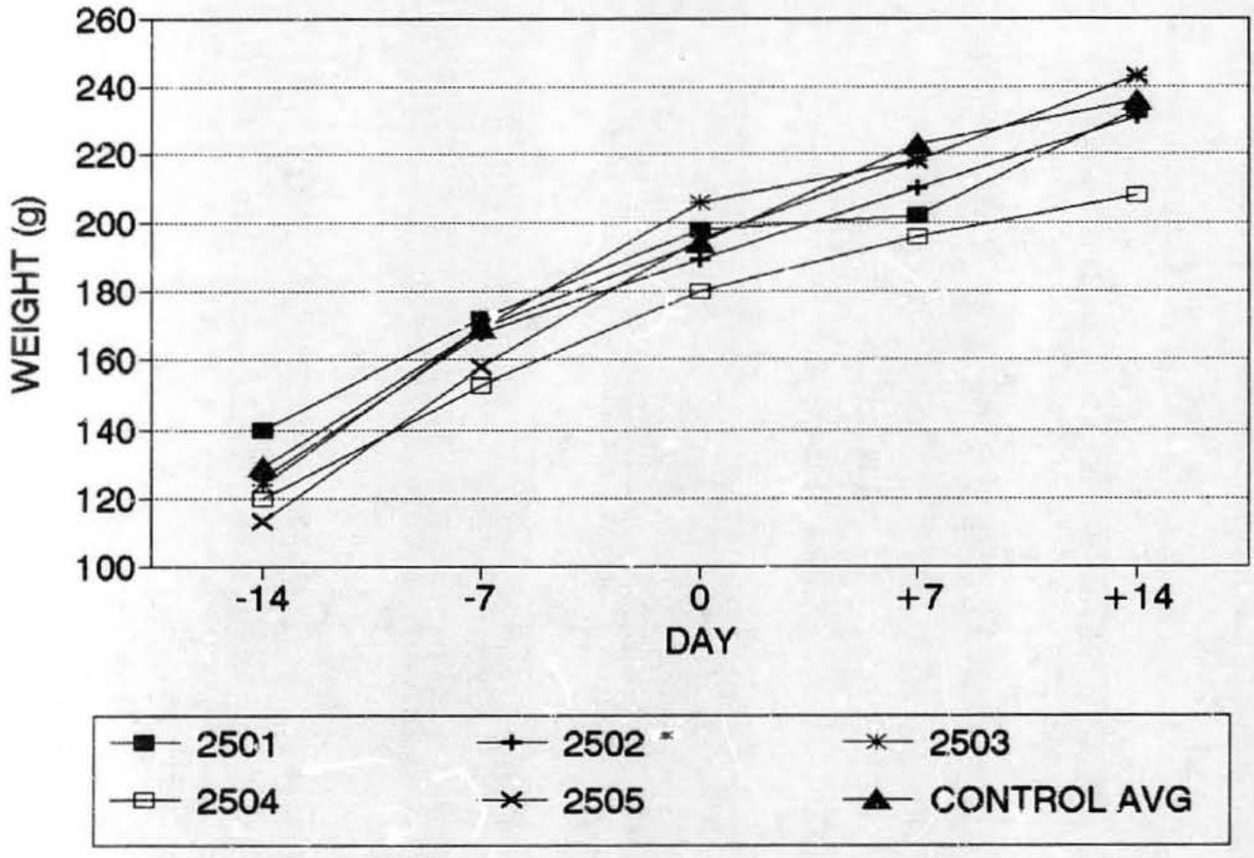
GROUP II: [redacted] mg/kg/day



000008

FEMALE RAT BODY WEIGHT

GROUP II: [redacted] mg/kg/day



000009

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

[redacted]

[redacted]

JUN 04 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

5091100628

EPA-OTS

000793750V

Re: P89-632

Dear [redacted]:

The Environmental Protection Agency (EPA) has reviewed the submitted 2-week oral toxicity study in rats and the protocol for a 90-day toxicity study by the oral route on the chemical substance described in premanufacture notice (PMN) P-89-632.

EPA has reviewed the data from the 2-week study and has determined that [redacted] has conducted the study in good faith and with due care in a scientifically valid manner. The report, received on April 16, 1991, demonstrates lethality in the mid- and high-dose groups of 20% and 60%, respectively, with dose levels set at 375, 750 and 1,500 mg/kg/day. In general, survival rate and reduction in body weight gain were also related to dose. There were indications of hepatotoxicity in all treated groups, renal toxicity was observed in mid-dose males with reduced kidney weight and high-dose males and females had increased blood ureanitrogen. Effects on lymphoid tissue were apparent in the mid- and high-dose groups with reduced blood lymphocytes and the high-dose group also showed reduced splenic weights and microscopic evidence of lymphoid atrophy. The report considers the low-dose to be a NOEL, however the serum enzyme changes and reduced body weight suggest some mild toxicity at this dosage level.

EPA's comments and recommendations on the proposed 90-day study protocol are designed to avoid potential problems so that the results of the study will be scientifically valid test data. Under no circumstance does approval of the test protocol mean pre-acceptance of test results. The following are EPA's comments on the study:

o In view of the findings in the 2-week range-finding study, EPA concurs with the suggested dosage levels for the 90-day oral toxicity study: 25, 75, 250 mg/kg/day.

o Although probably a mere photocopying issue, there is text missing from the bottom of several pages including pages 3, 4, 14, 15 and 16. There did not seem to be any critical details omitted which would affect the outcome of the study.

CONCURRENCES

SYMBOL	TS 794	D-296	TS-794					
SURNAME	Birnbaum	Kent	Allen-ISI					
DATE	5/29/91	6/3/91	6/4/91					

Printed on Recycled Paper

EPA Form 1320-1A (1/90)

Printed on Recycled Paper

OFFICIAL FILE COPY

000010

If you have any questions or comments, please contact Rona Birnbaum, the Program Manager assigned to this PMN, at (202) 245-4142.

Sincerely,

Rose Allison
Section Chief
New Chemicals Branch

[redacted]

91 APR 30 AM 7:53

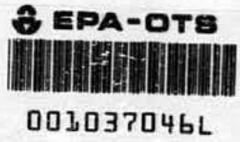
[redacted]

April 16, 1991

R.B. 4/16/91

50-9180000739

Ms. Rona Birnbaum
Office of Toxic Substances
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460



RE: PMN SUBSTANCE P-89-632

Dear Ms. Birnbaum:

On behalf of the [redacted], I am pleased to submit the final report of the two-week range finding study for PMN substance P-89-632. As we discussed, [redacted] would appreciate receiving EPA concurrence on the dose levels selected for the 90-day study which is being conducted pursuant to TSCA Section 5(e) Consent Order ([redacted] [redacted].

In the range-finding study three groups of animals were exposed for 14 days at dose levels of 1,500, 750 and 375 mg/kg/day. Deaths were reported at the dose levels of 1,500 and 750 mg/kg/day as well as other treatment related effects in surviving animals at these levels. The study report classifies the low dose of 375 mg/kg/day as the No Effect Level (NOEL) for this study.

In selecting dose levels for the 90-day study, we initially considered 375 as the upper dose. However, a careful analysis of the 14-day study suggests that although there were no deaths at this dose level early suggestions of toxicity were observed which cause us to believe that the 375 mg/kg/day level may be excessive as the high dose for the longer 90-day study. Specifically, there appears to be a decrease in body weight of more than 10% for two males at the 375 mg/kg/day level; thymic congestion was also reported for one of these males.

The TSCA guidelines for the 90-day oral toxicity study (40 CFR § 798.2650) states that the "highest dose level in rodents should result in toxic effects but not produce an incidence of fatalities which would prevent meaningful evaluation." Based on the reported findings in the 14-day study, we are concerned that dosing the animals at 375 mg/kg/day for 90 days may produce mortality and/or other toxic manifestations in excess of what is

[redacted]

generally considered acceptable for 90-day studies. It is therefore our intention to select 250 mg/kg/day as the upper dose for the study. We anticipate that this level will induce some adverse effects following 90 days of exposure and avoid loss of animals. For the low dose we are selecting 25 mg/kg/day to span one log unit and for the mid dose, 75 mg/kg/day, which is roughly halfway between the high and the low doses on a log scale.

Given that the 90-day study is being conducted pursuant to a TSCA Section 5(e) Consent Order we would like to receive the Agency's concurrence on these dose selections. Since [redacted] desires to start the 90-day study as soon as possible we would appreciate receiving a response from EPA within two weeks. Please let me know if you will be unable to respond within this time frame.

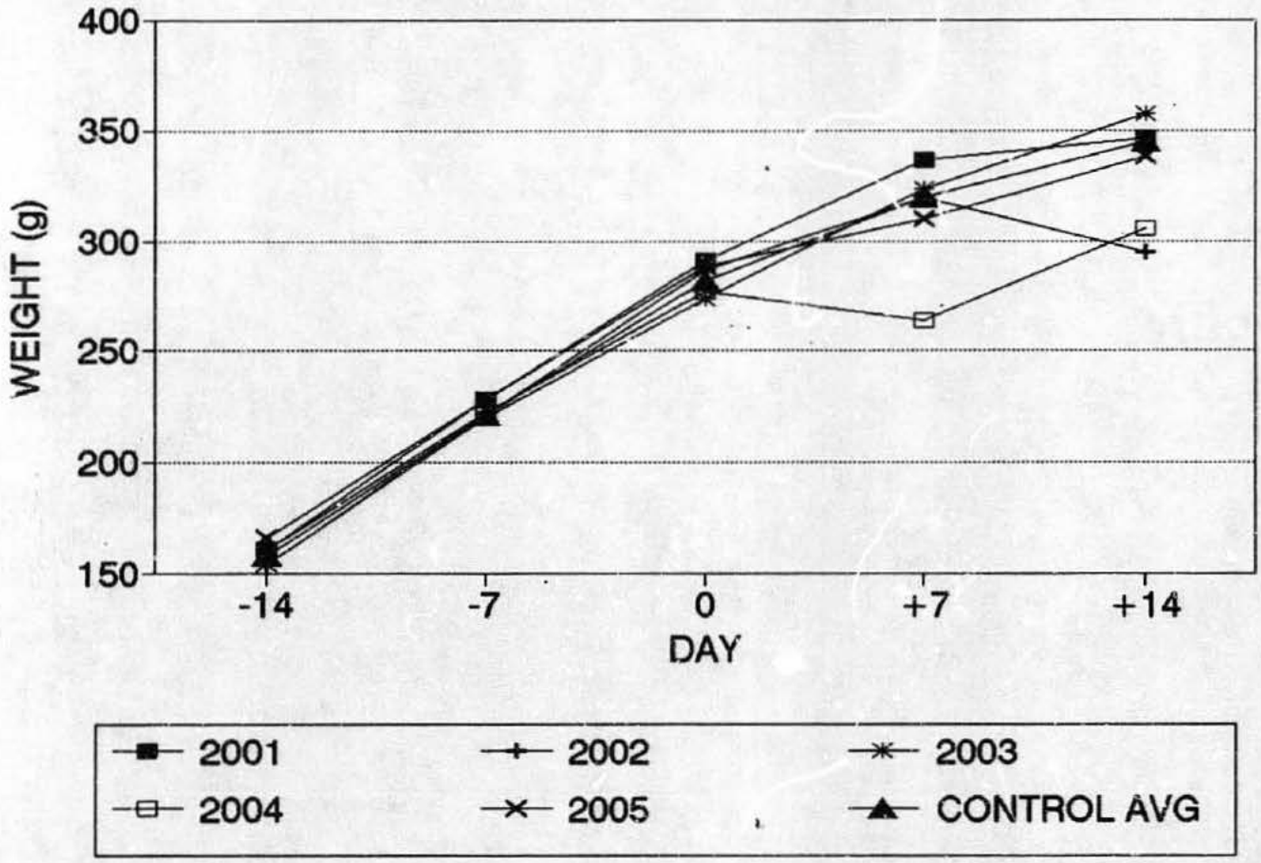
Sincerely,

[redacted]

cc: [redacted]

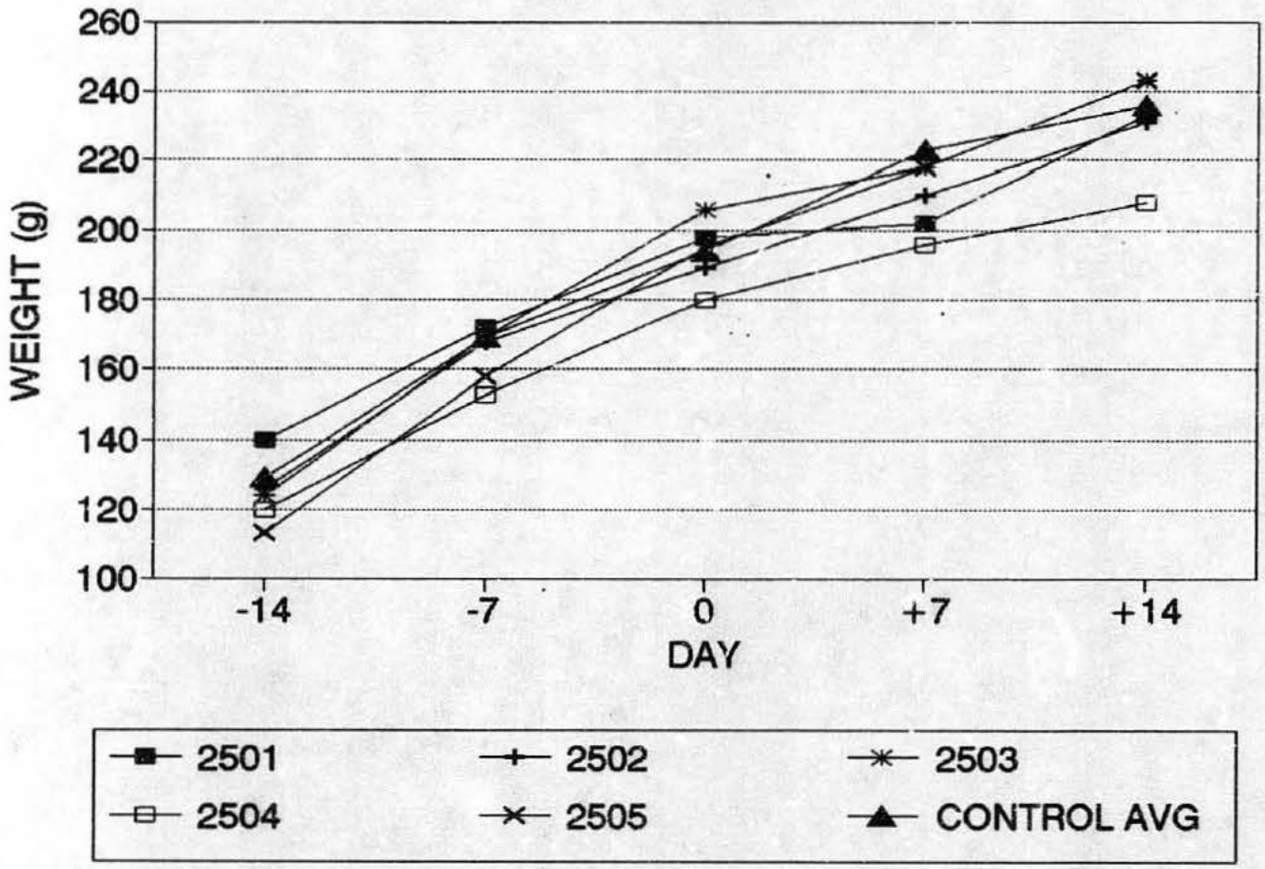
MALE RAT BODY WEIGHT

GROUP II: [redacted] mg/kg/day



FEMALE RAT BODY WEIGHT

GROUP II: [redacted] mg/kg/day



[redacted]

[redacted]

PROJECT NO. [redacted]

A 2 Week Oral Toxicity Study

of [redacted] in the Rat via Oral Gavage Administration

Final Report

Submitted to: [redacted]

Attn:

Date: 9 April 1991

[redacted]

[redacted]

[redacted]

PROJECT NO. 90-3588

A 2 WEEK ORAL TOXICITY STUDY

OF [redacted] IN THE RAT VIA ORAL GAVAGE ADMINISTRATION

ABSTRACT

This study, conducted for [redacted], was designed to assess the potential toxicity of [redacted] when administered orally via gavage to 30 Sprague Dawley CD® rats (5/sex/group) at dose levels of 375, 750 and 1500 mg/kg/day (Groups II, III and IV, respectively) for a period of 2 weeks. Control animals, Group I (5/sex/group) received the vehicle at the same dose volume as administered to the treated animals.

Physical observations, body weight and food consumption measurements were performed on all animals pretest and at selected intervals during the treatment period. In addition, hematology, clinical chemistry and urinalysis parameters were performed on all surviving animals prior to study termination.

After 2 weeks of treatment, all survivors were sacrificed, selected organs were weighed and organ/body and organ/brain weight ratios calculated. Complete gross postmortem examinations were conducted on all animals. Histopathological evaluation of selected tissues were conducted on animals in Groups I, III and IV; for Group II, the spleen, thymus and lymph nodes only were evaluated.

[redacted] was toxic at 750 and 1500 mg/kg/day but not at 350 mg/kg/day. Toxic manifestations seen included mortality (one male and one female in Group III; three males and three females in Group IV), physical observations indicative of toxicity, decreases in body weights and decreases in food consumption.

There was an increase in segmented neutrophils but a decrease in lymphocytes as determined by differential counts and these changes were the most pronounced in Group III and Group IV males. Also, Group III and IV animals had increased aspartate aminotransferase and alanine aminotransferase but decreased total protein and albumin. Group IV also had increased blood urea nitrogen (BUN), creatinine and BUN/creatinine ratios. Males but not females had decreased liver and kidney weights. Adrenal weights in both sexes were increased. Group IV animals had treatment-related decreased spleen sizes and lymphoid atrophy.

Since no effects were seen in the 375 mg/kg/day dose group, this dose is considered the no effect dose level for the study.

[redacted]

[redacted]

TABLE OF CONTENTS

[redacted]

TABLE OF CONTENTS (CONT.)

[redacted]

APPENDICES

I. INTRODUCTION:

This study, conducted for [redacted], w[redacted] the potential toxicity of [redacted] when administered orally via gavage to 30 Sprague-Dawley CD® rats (5/sex/group) at dose levels of 375, 750 and 1500 mg/kg/day (Groups II, III and IV, respectively) for a period of approximately 2 weeks. Control animals, Group I (5/sex/group) received the vehicle at the same dose volume as administered to the treated animals.

Species and strain of test animal, method and route of test substance administration and dose levels were determined by the sponsor. This study was conducted at [redacted] All raw data, specimens, the original study protocol, the original final report and a sample of the test substance are stored in the Archives of [redacted]

[redacted]

II. MATERIALS AND METHODS:

A. Test Substance: [redacted]

Supplier: [redacted]

Lot No.: [redacted]

Concentration: [redacted] Active Ingredient

Description: [redacted]

Dates Received: 18 July 1990 (Container: 1)
02 August 1990 (Container: 2)

Analysis: The identity, strength, purity and composition; and synthesis, fabrication, and/or derivation of the test substance have been documented by the sponsor.

Stability: The stability of the test substance has been determined by the Department of Analytical Chemistry at [redacted]

Storage: In tightly sealed, light-resistant containers in a temperature monitored room (60-85°F; 16-29°C).

Sampling: An archival sample of approximately 10 grams of test substance is stored in the Archives of [redacted]

Disposition: All remaining containers of the test substance will be returned to the sponsor after completion of further testing.

B. Vehicle: Methylcellulose (as a 0.5% aqueous solution)

Description: Fine white powder

Lot Number: [redacted]

Storage: Room temperature, keep dry.

C. Test Animals: Rats

Strain: CD® (Sprague-Dawley derived)

[redacted]

II. MATERIALS AND METHODS (cont.):

C. Test Animals (cont.):

Justification for Animal Selection:	The rat is a rodent animal model commonly utilized in toxicity studies as recommended in the referenced guidelines. In addition, a historical data base is available for comparative evaluation.
Number of Animals:	
Received:	63 total (32 males, 31 females)
Placed on Test:	40 total (20 males, 20 females)
Supplier:	[redacted]
Date Received:	16 August 1990
Age at Receipt:	28 Days old.
Age at Initiation of Treatment:	50 Days old.

Weight at Initiation of Treatment (grams):

	Mean	Range
Males:	282.5	272.5-294.2
Females:	193.4	179.1-212.0

Acclimation Period: Animals were acclimated for approximately 3 weeks (16 August to 6 September 1990). All animals were examined by the staff veterinarian during the acclimation period.

D. Selection: More animals than required for the study were purchased and equilibrated. Animals considered unsuitable for the study on the basis of pretest physical examinations and/or outlying body weight data were eliminated prior to random selection for group assignment.

E. Group Assignment: Animals considered suitable for study were distributed into 4 groups of 5 animals per sex by a computerized random sort program in an attempt to equalize mean group body weights. Groups were assigned to control and dose levels randomly.

II. MATERIALS AND METHODS (cont.):

F. Animal Identification:

Each rat was identifi[redacted] tag bearing its uniqu[redacted] Inc. animal number. [redacted] lost, it was replaced[redacted] each cage was provided with a cage card which was color coded for dose level identification and contained the project number, animal number, sex and dose-group information.

G. Experimental Outline:

Group	Dose Level[a] (mg/kg/day)	Number of Animals: Initial M	Initial F	Clinical Laboratory Studies Termination M	Clinical Laboratory Studies Termination F	Terminal[b] Sacrifice M	Terminal[b] Sacrifice F	Histopathology[c] M	Histopathology[c] F
I	0	5	5	5	5	5	5	5	5
II	375	5	5	5	5	5	5	-	-
III	750	5	5	5	5	5	5	-	-
IV	1500	5	5	5	5	5	5	5	5

[a]Control animals received vehicle in the same volume administered to the test animals.
[b]Gross necropsy examinations were performed on all animals which died prior to study termination.
[c]Histopathological evaluations were performed on all animals in the control, mid- and high-dose groups; for the low-dose group, the spleen, thymus and lymph nodes only were evaluated.

H. Husbandry:

Housing: Animals were doubly housed in elevated stainless steel wire mesh cages during the first week of the acclimation period and individually housed thereafter.

Food: ad libitum; standard laboratory diet (Purina Certified Rodent Mash-type Diet® #5002). Fresh food presented weekly.

000023

II. MATERIALS AND METHODS (cont.):

H. Husbandry (cont.):

Analysis of Feed:	Analysis of each feed lot used during this study was performed by the Purina Mills® Company prior to receipt at [redacted] Results are maintained on file at [redacted]
Water:	ad libitum; by automated watering system [redacted]
Analysis of Water:	Water analysis was provided by [redacted] Plant). Results are maintained on file at [redacted]
Environmental Conditions:	12 hour light/dark cycle (7 AM to 7 PM) via automatic timer; temperature and humidity monitored and recorded twice and once daily, respectively.
Temperature:	Desired: 67-76°F (19-24°C) Actual: 67-74°F (19-23°C)
Humidity:	Desired: 40-60% Actual: 45-83%

I. Test Substance Administration:

Route:	Oral via intubation (oral gavage)
Justification of Route of Administration:	The oral route is one of the potential routes of human exposure to this test substance.
Homogeneity and Stability:	Prior to initiation of study, dose solutions were prepared for the low- and high-dose groups (Groups II and IV, respectively) for homogeneity and stability analysis. Analyses were performed by the Department of Metabolsim and Analytical Chemistry at [redacted] Results are presented in Appendix K.

000024

[redacted]

II. MATERIALS AND METHODS (cont.):

I. Test Substance Administration (cont.):

Method:	Appropriate amounts of test substance were suspended in the vehicle weekly to yield dose levels of 375, 750 and 1500 mg/kg/day at a constant dose volume of 10 ml/kg/dose. Individual doses were adjusted by most recent weekly body weight. Control animals were administered the vehicle at the same dose volume.
Dose Volume:	10 ml/kg/dose
Frequency:	Once daily seven days a week.
Duration:	14 days
Dates of Treatment:	7 September to 20 September 1990
Sampling:	Samples of all three dose levels were taken weekly throughout the study period for analysis of dose level concentration. Analyses performed by [redacted] Results presented in Appendix K.

J. Observations:

For Mortality and Gross Signs of Toxicologic or Pharmacologic Effects:	Twice daily, once in the morning and once in the afternoon.
Detailed Physical Examination for Signs of Local or Systemic Toxicity, Pharmacologic Effects and Palpation for Tissue Masses:	(Methodology and References, Appendix A) Pretest and weekly thereafter.
K. Body Weight:	(Methodology and References, Appendix A) Three times pretest and weekly during treatment.

[redacted]

000025

[redacted]

II. MATERIALS AND METHODS (cont.):

L. Food Consumption: (Methodology and References, Appendix A)

Weekly, beginning two weeks prior to treatment and weekly (every 6 days) throughout the study.

M. Laboratory Studies: (Methodology and References, Appendix A)

Blood was obtained via venipuncture of the orbital sinus (retrobulbar venous plexus) under light ether anesthesia. Rats were fasted overnight prior to blood collections.

Number of Animals: Performed on up to 5 animals/sex/group at study termination.

Parameter Evaluated	Time Intervals
Hematology:	
hemoglobin concentration hematocrit erythrocyte count reticulocyte count platelet count mean corpuscular volume mean corpuscular hemoglobin mean corpuscular hemoglobin concentration prothrombin time activated partial thromboplastin time total and differential leukocyte counts erythrocyte morphology	Termination: 21 September 1990

[redacted]

000026

[redacted]

II. MATERIALS AND METHODS (cont.):

M. Laboratory Studies (cont.):

Parameter Evaluated

Clinical Chemistry:	Time Intervals
aspartate aminotransferase	Termination: 21 September 1990
alanine aminotransferase	
alkaline phosphatase	
blood urea nitrogen	
fasting glucose	
cholesterol	
bun/creatinine ratio	
total protein	
albumin	
globulin (calculated)	
A/G ratio (calculated)	
creatinine	
total bilirubin	
sodium	
potassium	
chloride	
calcium	
phosphorus	
gamma glutamyl transpeptidase	

Urinalysis:	Time Intervals
gross appearance	Termination: 20 September 1990
specific gravity	
pH	
protein	
glucose	
ketones	
bilirubin	
occult blood	
16-hour volume	
microscopic examination of sediment	

[redacted]

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[redacted]

II. MATERIALS AND METHODS (cont.):

[redacted]

N. Postmortem:

Animals Found Dead or Killed at Terminal Sacrifice:	Complete gross postmortem examinations were performed on all animals. External surface, all orifices, the cranial cavity, carcass, the external and surface of the brain and spinal cord, the thoracic, abdominal and pelvic cavities and their viscera and cervical tissues and organs were examined for all animals. Animals were fasted prior to scheduled sacrifices.
Necropsy:	21 September 1990
Number of Animals:	32 animals total.
Sacrifice Method:	Exsanguination under carbon dioxide anesthesia.
Organs Weighed and Organ/Body and Organ/Brain Weight Ratios Calculated:	(Methodology and References, Appendix A)

The following organs were weighed for all animals at the scheduled sacrifice intervals. Paired organs were weighed together.

brain
adrenals
kidneys
testes with epididymides
thyroid/parathyroids
liver
ovaries

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[redacted]

II. MATERIALS AND METHODS (cont.):

N. Postmortem (cont.):

[redacted]

Tissues Preserved:[1]

adrenals (2)
brain
epididymides (2)
eyes
heart
intestine
 duodenum
kidneys (2)
liver (2 sections)
lungs
lymph nodes (mesenteric and mediastinal)
ovaries
spleen
sternum (with bone marrow)
stomach
testes (2)
thymus
thyroids/parathyroids
urinary bladder
uterus (2)
gross lesions

Tissues Examined Histopathologically:[2]

All tissues listed below were examined for all animals in the control, mid- and high-dose groups; for the low-dose group, the spleen, thymus and lymph nodes only were evaluated.

adrenals (2)
brain
epididymides (2)
eyes
heart
intestine
 duodenum
kidneys (2)
liver (2 sections)
lungs
lymph nodes (mesenteric and mediastinal)
ovaries
spleen
sternum (with bone marrow)
stomach
testes (2)

[1]Number in parentheses indicates number of organs/sections preserved.
[2]Number in parentheses indicates number of organs/sections examined.

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[redacted]

II. MATERIALS AND METHODS (cont.):

N. Postmortem (cont.):

[redacted]

Tissues Examined Histopathologically (cont.):[2]

thymus
thyroids/parathyroids
urinary bladder
uterus (2)
gross lesions

Preservatives: 10% neutral buffered formalin (testes and epididymides were placed in Bouin's solution for the initial 48-72 hours, transfered to 70% Synosol® and then preserved in formalin).

Stain: (Methodology and References, Appendix A)

Hematoxylin and Eosin

O. Statistical Analysis: (Methodology and References, Appendix A)

Body weight, body weight change from Week 0, food consumption, hematology and clinical chemistry parameters, terminal organ weights and body weights and organ/body and organ/brain weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval. Statistically significant differences from control are indicated on mean tables of appendices.

P. Protocol Deviations: The following protocol deviations occurred during the study period. None of the deviations are considered to have had an adverse effect on the study purpose or results.

1. Humidity deviated from the desired range on some occasions.
2. Urinalysis Bilirubin values were not required as per protocol but were recorded as a result of technician error.

[2]Number in parentheses indicates number of organs/sections examined.

[redacted]

III. RESULTS AND DISCUSSION:

A. Mortality (Appendix B):

There was no mortality in the Group I (vehicle control) or Group II (375 mg/kg/day) animals. In Group III (750 mg/kg/day), one male died on Day 5 and one female animal died on Day 8. Finally, in Group IV (1500 mg/kg/day), three males died (one each on Days 3, 6 and 12) and three females died (all on Day 4).

B. Physical Observations (Appendix C):

In all of the animals which died before the terminal sacrifice, a general appearance of lethargy, dyspnea, stains on snout, decreased fecal volume, ano-genital staining, soft stool, decreased fecal volume and decreased food consumption were observed. Observations in animals which survived were of the type commonly seen in laboratory rats.

C. Body Weights (Appendix D):

There were statistically significant decreases in body weights and weight gains in Group III males during Weeks 1 and 2 of the study. There were also decreases (though not statistically significant) in the Group II and Group IV males.

D. Food Consumption (Appendix D):

Week 1 food consumption for Group III males was statistically significantly decreased, relative to the control values. Week 2 food consumption was statistically significantly increased for Group IV females. These do not appear to be adverse effects of test substance administration.

[redacted]

III. RESULTS AND DISCUSSION (cont.):

E. Laboratory Studies:

1. Hematology (Appendix E and Appendix F):

For Groups III and IV, the lymphocytes were generally decreased and the segmented neutrophils were increased, as determined by differential leukocyte counts. These effects were dose and treatment related and were more pronounced in males.

2. Clinical Chemistry (Appendix G):

The Group III and Group IV animals had increased aspartate aminotransferase (SGOT) and alanine aminotransferase (SGPT) but decreased alkaline phosphatase, total protein, albumin and chloride values. The Group IV animals also had increased blood urea nitrogen (BUN), creatinine and BUN/creatinine ratios. These effects were treatment related.

3. Urinalysis (Appendix H):

Test substance administration did not affect the urinalysis parameters evaluated.

F. Terminal Organ and Body Weights and Organ/Body and Organ/Brain Weight Ratios (Appendix I):

In males, but not females, there were treatment-related decreases in the kidney and liver weights in the Group III animals. Also, the Group III females and Group IV males and females had a trend of increasing adrenal weights with increasing dose.

G. Pathology (Appendix J):

The primary, treatment-related pathologic findings were decreased spleen sizes in 2 of 5 males and 4 of the 5 females in Group IV. There was also lymphoid atrophy in one of the males and all of the females with the decreased spleen sizes.

III. RESULTS AND DISCUSSION (cont.):

H. Dose Solution Analysis (Appendix K):

[redacted] was found to mix homogeneously in 0.5% methylcellulose and was found to be stable for 14 days refrigerated. In addition, actual dose suspensions administered to the animals were within an acceptable range from nominal (± 15%).

IV. CONCLUSION:

The administration of [redacted] by oral gavage at doses of 750 mg/kg/day or 1500 mg/kg/day to rats caused a dose-related mortality along with toxic manifestations in the various parameters measured in this study. In contrast, administration of [redacted] at a dose of 375 mg/kg/day did not induce toxicity in the rats in this study, and hence, is considered the no effect level dose.

[redacted]

Figure 1
A 2 Week Oral Toxicity Study
of [redacted] in the Rat via Oral Gavage Administration

Group Mean Body Weight in Male Rats

[redacted]

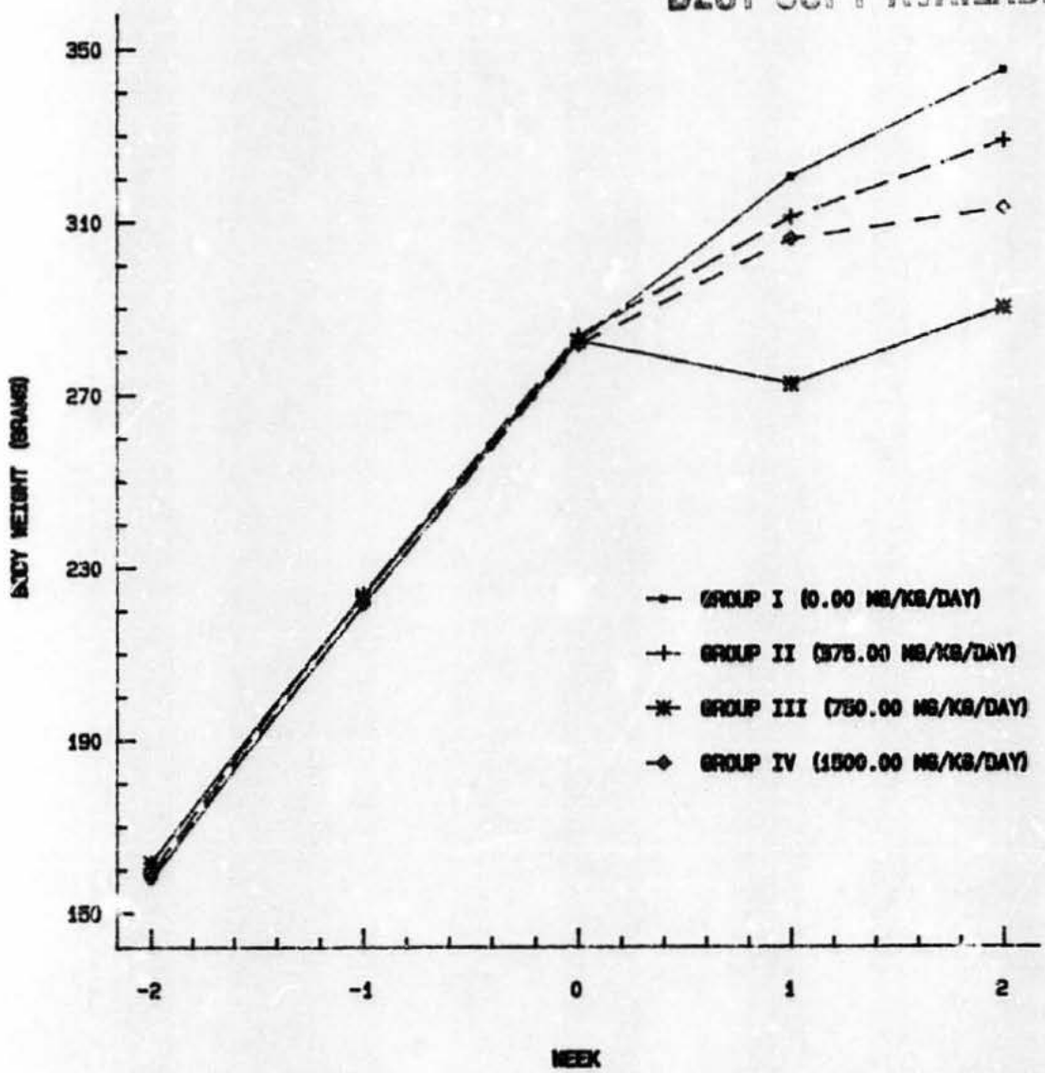

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Figure 2

of [redacted] A 2 Week Oral Toxicity Study in the Rat via Oral Gavage Administration

Group Mean Body Weight in Female Rats

[redacted]

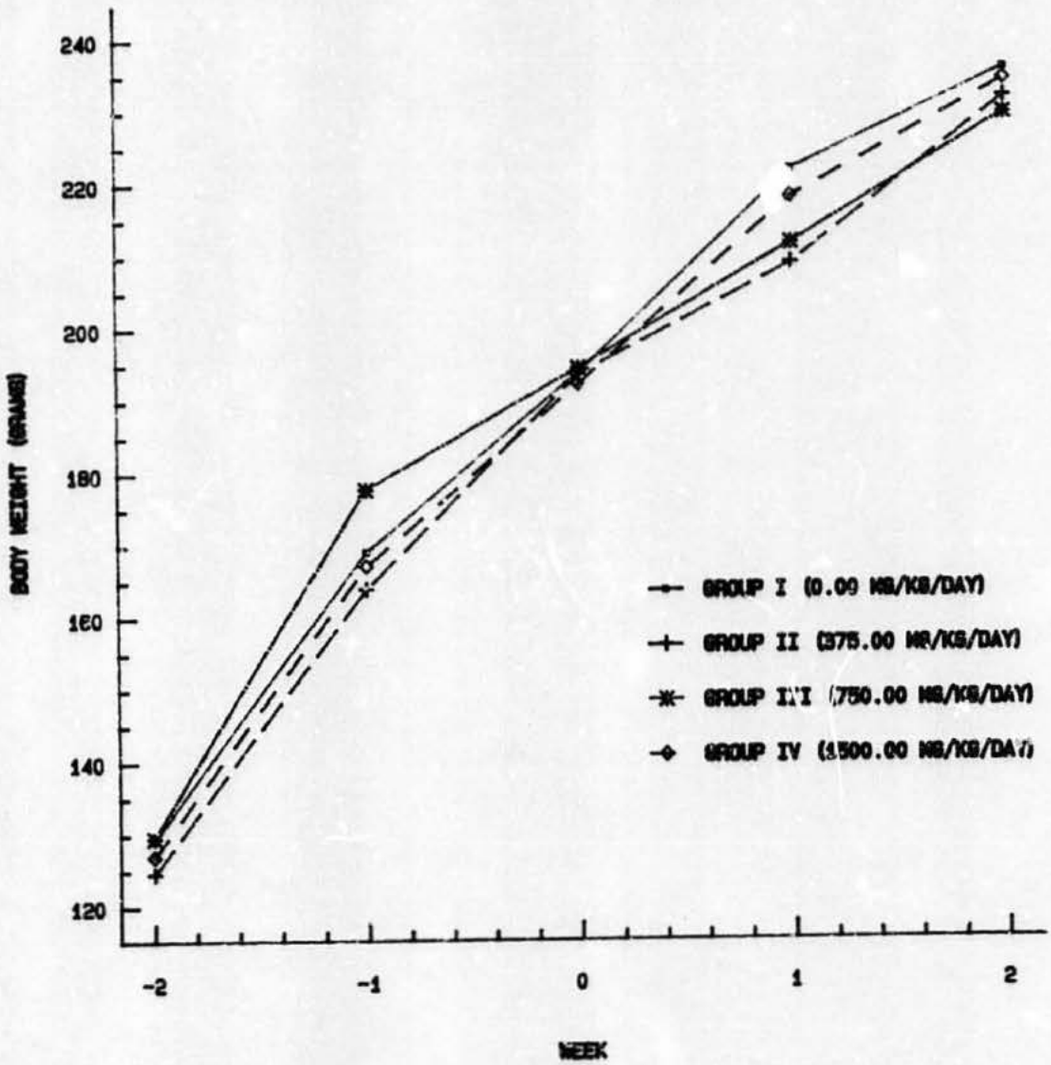

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Figure 3

A 2 Week Oral Toxicity Study

of [redacted] in the Rat via Oral Gavage Administration

Group Mean Food Consumption in Male Rats

[redacted]

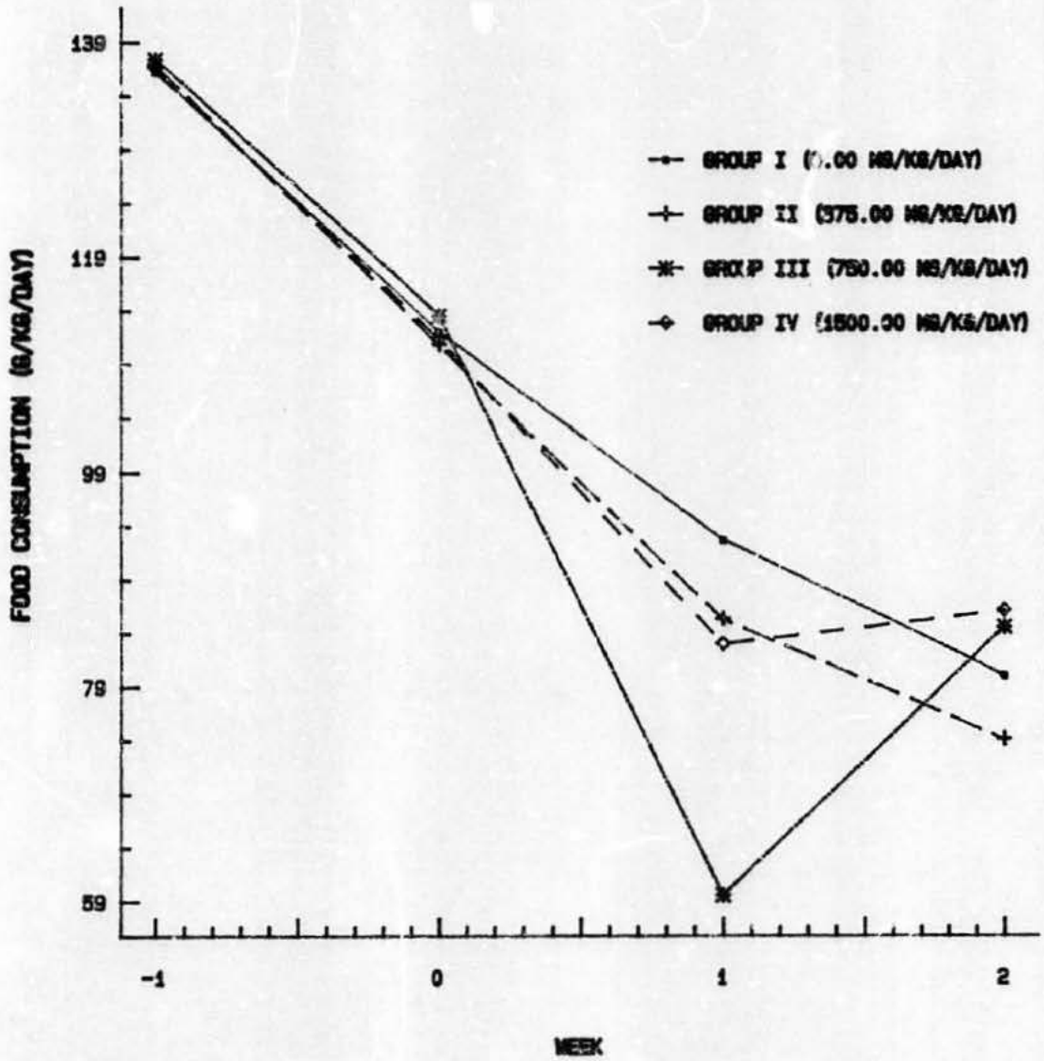

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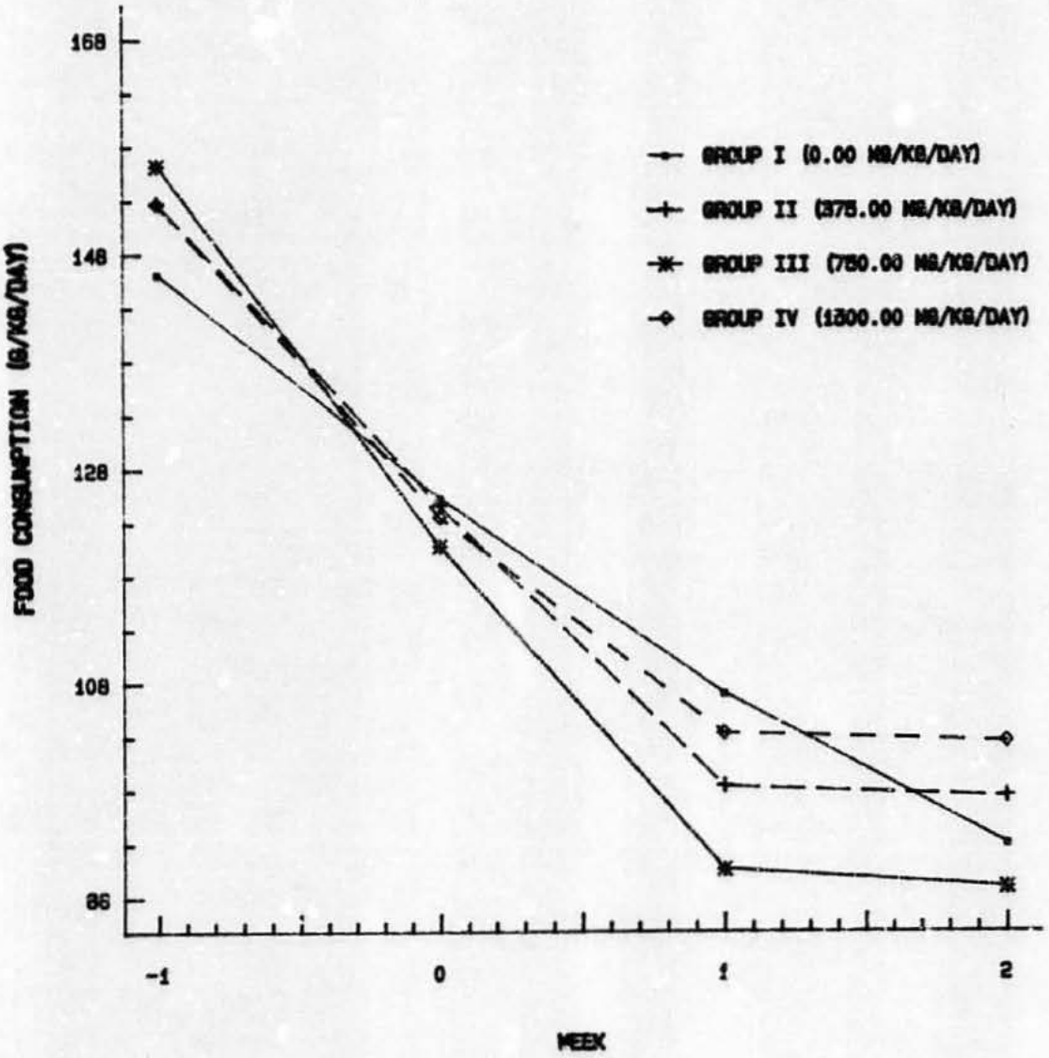
Figure 4
A 2 Week Oral Toxicity Study
of [redacted] in the Rat via Oral Gavage Administration

Group Mean Food Consumption in Female Rats

[redacted]



Appendix A
A 2 Week Oral Toxicity Study
of [redacted] in the Rat via Oral Gavage Administration

Methodology and References - General

Parameter	Reference or Description
Physical Examination	Behavior: aggressiveness, increased or decreased activity. Respiration: nasal discharge and rales. Ocular: chromodacryorrhea, excessive lacrimation and percent opacity. Appearance: alopecia, ano-genital staining and general condition. Gastrointestinal: abdominal shape and fecal consistency. Palpation for tissue masses. Other: includes any unusual observation not included above.
Body Weight	Sartorius Universal Electronic Toploading Balance, Model U3600.
Food Consumption	Sartorius Universal Electronic Toploading Balance, Model U3600. Feed was available ad libitum 7 days/week. Animals were presented with full feeders weighing 570 grams (includes weight of feed, jar and lid). After 6 days feeders were reweighed and resulting weight was subtracted from the full feeder weight. Resulting value = g/6 days (g/interval). $g/kg/day = \frac{g/interval}{average\ body\ weight\ (kg)} \div 6\ days.$ $Average\ BW = \frac{Previous\ BW + Current\ BW}{2}$
Terminal Body Weight (TBW)	Ohaus B 5000. Represents a fasted body weight measured just prior to necropsy.
Organ Weights	Mettler AK-160 - All organs.
Histological Methods	
Stain - Hematoxylin and Eosin	Sheehan, D.C., and Hrapchak, B.B., Theory and Practice of Histotechnology. 2nd Edition. Columbus: Battelle Press, 1987, pp.143-144.

Appendix A (cont.)
A 2 Week Oral Toxicity Study
of [redacted] in the Rat via Oral Gavage Administration

Methodology and References - Hematology

Abbreviation	Parameter	Specimen	Reporting Units	Reference or Description
HGB	Hemoglobin Concentration	Whole Blood	g/dl	Technicon® H-1™ Hematology System, Technicon Instruments Corporation.
HCT	Hematocrit	Whole Blood	percent	Technicon® H-1™ Hematology System, Technicon Instruments Corporation.
RBC	Erythrocyte Count	Whole Blood	10^6/microliter (mil/μl)	Technicon® H-1™ Hematology System, Technicon Instruments Corporation.
RETIC	Reticulocyte Count	Whole Blood	% RBC	New Methylene Blue stain. Determined by manual microscopy.
PLAT	Platelet Count	Whole Blood	10^5/microliter (100 T/μl)	Technicon® H-1™ Hematology System, Technicon Instruments Corporation.
MCV	Mean Corpuscular Volume	Whole Blood	cubic μ	Technicon® H-1™ Hematology System, Technicon Instruments Corporation.
MCH	Mean Corpuscular Hemoglobin	Whole Blood	$\mu\mu$g	Technicon® H-1™ Hematology System, Technicon Instruments Corporation.
MCHC	Mean Corpuscular Hemoglobin Concentration	Whole Blood	g/dl	Technicon® H-1™ Hematology System, Technicon Instruments Corporation.
PT	Prothrombin Time	Plasma	seconds	Photo-optical clot detection system, COAG-A-MATE® X2. General Diagnostics Division, Division of Warner-Lambert Company.
APTT	Activated Partial Thromboplastin Time	Plasma	seconds	Photo-optical clot detection system, COAG-A-MATE® X2. General Diagnostics Division, Division of Warner-Lambert Company.

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